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Original Research Article

EVALUATION OF PHYTOCHEMICAL AND BIOLOGICAL PROPERTIES ON *STROBILANTHES CILIATUS* NEES

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ABSTRACT

The present study was carried out to evaluate the phytochemical and biological properties of the plant of *Stobilanthes ciliatus* Nees. In this study, qualitative and quantitative determinations were conducted by different methods. The quantitative studies revealed the total percentage content of steroidal saponins, flavanoids, tannins and terpenoids in the leaves, stem and root respectively. Cytotoxic property of the plant extract was studied against human breast cancer (MCF-7) cell lines using thiazolyl blue dye exclusion technique (MTT assay). The extract showed moderate *in vitro* cytotoxic activity having IC_{50}value of 3.68µg/ml. Analgesic property of the plant extract at a dose of 100 mg/kg & 200 mg/kg was evaluated against the standard drug Pentazocin at a dose of 5 mg/kg using tail clip models of pain. The extract of *Stobilanthes ciliatus* Nees showed more significant analgesic activity ($p<0.001$) as compared to standard drug.

Key Words: *Stobilanthes ciliatus* Nees, Phytochemical studies, *In vitro* Cytotoxic study, *In vivo* Analgesic study, Tail clip method.

INTRODUCTION

The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicines irrespective of the underlying philosophical premise. Plants have been used in the treatment of various diseases from time immemorial and are the only economic source of a number of well-established and important drugs. It is interesting to note that the most significant therapeutic agents introduced to medicine within recent years have not been of synthetic origin but have been obtained from natural sources. Knowledge of the medicinal plants in traditional systems of medicine (TSM) used as a lead for the discovery of new medicines for the modern system of medicine[1].Among the plants of Kolli Hills, *Strobilanthes ciliatus* Nees belongs to the family Acanthaceae have got high reputation in traditional medicinal practice for its remarkable medicinal properties. The entire plant was recognized as valuable drug and frequently used by many of the ancient traditional medical systems. The leaves, stem, seed and roots of the plant is to possess a number of therapeutic effects such as diuretic, diaphoretic, anti-inflammatory, lumbago, sciatica, limping, chest congestion, jaundice, dropsy, urogenital infections, leprosy, diabetics, fever, leucoderma, skin diseases, cough, bronchitis, odontalgia and treatment of rheumatism[2,3]. Numerous interesting secondary metabolites such as saponin glycosides, sterols, tannins, flavonoids and terpenoids were reported for the genus of *Strobilanthes ciliatus* Nees. Earlier investigations

on the plant *Strobilanthes ciliatus* Nees have lead to the isolation of lupeol, stigmasterol, betulin, taraxerol and 4-acetyl-2,7-dihydroxy-1,4,8-triphenyloctane-3,5-dione[4]. The acetone and ethanolic extracts of *Strobilanthes ciliatus* have been evaluated for their antimicrobial, antioxidant and cytotoxic properties[5,6]. In spite of the widespread use of *Strobilanthes ciliatus* Nees and phytoconstituent reported, there hardly exists any documentation on the pharmacological profile of the plant. Hence, in the present study an attempt was made to prove the phytochemical and biological potential of the plant *Strobilanthes ciliatus* Nees.

MATERIALS AND METHODS

Collection, authentication and processing of plant material

Healthy plant of *Strobilanthes ciliatus* Nees was collected from the Western Ghats of Kolli Hills, Namakkal District, Tamilnadu State, India. After identification and authentication, fresh plant material was collected in bulk, washed under running tap water to remove adhering material, dried under shade and pulverized into coarse powder using a mechanical grinder.

Preparation of plant extract

The shade dried coarsely powdered drug of *Strobilanthes ciliatus* Nees was extracted with alcohol: water (80:20) at 60-70°C, until the extraction was completed. The successive hydroalcoholic extract was filtered and dried under reduced pressure to get a solid mass.

QUALITATIVE PHYTOCHEMICAL STUDIES

The term "Phytochemicals" according to the American Cancer Society refers to a wide variety of chemical compounds produced by plants that can be found in parts of the plant such as alkaloids, carbohydrates, glycosides, sterols, proteins, tannins, flavonoids, saponins and terpenoids. The hydroalcohlic extract of S*trobilanthes ciliatus* Nees was subjected to find different classes of phytoconstituents[7,8].

QUANTITATIVE PHYTOCHEMICAL STUDIES

Based on the qualitative phytochemical analysis, total percentage content of therapeutically important group of phytoconstituents was estimated by different methods. The total percentage content of steroidal saponins, tannins, flavonoids and terpenoids were estimated in leaves, root and stem of dried powdered drug respectively.

a. Estimation of total content of steroidal saponins[9] (Gravimetry method)

5.0 g of the powdered crude drug was accurately weighed and transferred to 100 ml round bottom flask, fitted with a reflux condenser. To this 25 ml of 90% methanol was added and refluxed for 30 minutes. The extraction process was repeated twice by using 25 ml of 90% methanol each time. All the extracts were collected and concentrated using a water bath. 25 ml of acetone was added drop wise to 10 ml of concentrated methanolic extract and precipitated. The acetone precipitate was filtered and concentrated to residue.

Total saponins = Wt. of residue x 100 x100 / Wt of sample x (100-LOD)

b. Estimation of total content of tannins[10] (Titrimetric method)

100 mg of powdered crude drug was accurately weighed and dissolved in 50 ml of distilled water, 750 ml of distilled water added and shaken well. To this 25 ml of Indigo carmine solution (0.6 g dissolved in 20 ml of concentrated sulphuric acid and made the volume to 400 ml with distilled water) was added and shaken well. Then the solution was titrated against N/10 Potassium Permanganate solution ($KMnO_4$) till a golden yellow color end point was attained. The experiment was repeated with the same quantity of reagents and in the same manner but omitting the substance. The differences between the two titrations represent the indigo carmine solution required to neutralize the tannin. Each ml of 0.1N $KMnO_4$ is equivalent to 0.004157 g of Tannin.

The percentage of Tannins was calculated (w/w) as:

(A-B) x 0.004157 x 100 x N / W x 0.1

A= Vol. of 0.1N $KMNO_4$ consumed in titration (Test Solution)

B=Vol. of 0.1N $KMNO_4$ consumed in titration (Blank Solution)

N =Normality of Potassium Permanganate (N/10)

W=Weight of the sample

c. Estimation of total content flavonoid[11-14] (Colorimetric Method)

Total flavonoid content was determined using quercetin as reference standard. Quercetin (100 mg) was dissolved in 10 ml methanol and diluted to provide a series of concentrations (5, 10, 25, 50 and 100 µg/ml). Accurately weighed 100 mg of powdered crude drug was transferred to 10 ml volumetric flask and made up the volume with methanol. 0.5 ml of both the standard and sample solution were added with 1.5 ml methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml potassium acetate 1M and 2.8 ml distilled water and incubated for 30 minutes. A calibration curve was made (Table No.2 & Figure No.1) by measuring the absorbance of the dilutions at 415 nm with a Shimadzu UV-1800 spectrophotometer. Distilled water with 10% AlCl3 was used as a blank. Total flavonoid content was expressed in gram equivalent Quercetin of each 100 g plant extract of dry weight.

d. Estimation of total content of terpenoids[15]

100 g of the powdered crude drug was soaked in alcohol for 24 hours, filtered and the filtrate was extracted with petroleum ether. The petroleum ether extract was treated as total terpenoids.

***IN VITRO* STUDIES**

Evaluation of Cytotoxic property

Principle[16,17]

The principle of this colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the water soluble yellow substrate 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically. Only viable cells with active mitochondria reduce the significant amount of MTT. The level of activity is measured by assessing the viable cells.

Media

Leibovitz L-15 Medium with L-Glutamine, FBS (Fetal bovine serum, SFM HEK-293 (serum free media), Thioglycollate medium (TGM), Tryptone soya broth (TSB) and cell proliferation kit (MTT) 1000 tests (Sigma Aldrich).

Cell lines

MCF-7 (Breast cancer cell line) purchased from NCCS, Pune.

Cell treatment procedure

Cytotoxic property of hydroalcoholic extract of *Strobilanthes ciliatus* Nees on the MCF-7 breast cancer cell lines was determined using the MTT proliferation assay kit. The cells (1×10 cells/ml) were preincubated in culture medium for 3 hrs at 37°C and 6.5% CO_2. The cells were seeded at a concentration of 5×104 cells/well in 100 µl culture medium and at various concentrations (5 -100 µg/ml) of standard Methotrexate and extract (dissolved in 2% DMSO (dimethyl sulphoxide) solution) into Microplates and incubated for 24 hrs at 37 °C and 6.5 % CO_2. The test denotes the surviving cells after toxic exposure. Then, 10 µl MTT labeling mixtures were added and incubated for 4 hrs at 37°C and 6.5% CO_2. Each experiment was done in triplicates. Followed by, 100 µl of solubilisation solution was added to each and incubated for overnight. The spectrophotometric absorbance of the samples was measured using a microplate (ELISA) reader at wavelength in between 550 and 600 nm. Percentage inhibition of extract against all cell lines was calculated using the following formula:

% of cell survival = AT/AC x 100

AT – Absorbance of test

AC – Absorbance of control (Cell)

% of cell inhibition = 100 - % of cell survival

The concentration required to inhibit 50% of cell viability (IC_{50}) was determined by plotting the log of the drug concentration versus the percentage of inhibition. The best - fit line was plotted by least-squares linear regression.

The 50% inhibitory concentration (IC_{50}) was calculated from the linear-regression equation: Log (CV_{50}) = m×log (IC_{50}) +c; where m is the regression coefficient, c is the intercept of the line, log (IC_{50}) is the log of the 50% inhibitory concentration of the extract and log (CV_{50}) is the log value of 50% cell viability[18].

***IN VIVO* STUDIES**

Acute oral toxicity studies[19]

Healthy *Wistar Albino* rats weighing 150-200 g were used for the acute toxicity study. The test doses were fixed with reference to the OECD Guidelines 423. All the animals were randomly distributed into one control group and three treated groups containing five animals per group. Groups I, II and III were orally administered plant extract of 100, 500 and 1000 mg/kg body weight respectively. The control group (Group IV) received vehicle alone. The test compounds were administered in a single dose not exceeding an ml by using oral gavage. The animals were observed continuously for each 4 hr to detect any changes in autonomic or behavioral responses such as spontaneous activity, irritability, corneal reflex, urination and salivation. Any mortality during the experimental period of 14 days was also recorded. The percentage in mortality in each group was noted.

Analgesic activity by tail clip method[20, 21]

The healthy adult *Swiss Albino* mice with the weight ranging from 20-25 g were divided into four groups each contains n=7 animals. The group I received only saline, Group II received Pentazocin 5 mg/kg, Group III and IV received 100 and 200 mg/kg body weight of hydroalcoholic extract respectively. The compounds administered before 15 min of the experiment and the artery clip were applied to the root of the tail approximately 1-2 cm to induce the pain. A sensitivity test was carried out and animals that were not attempted to dislodge the clip within 10 seconds were discarded. The noxious responds of the animals were noted (biting of the tail near the location of the clip) and the percentage of inhibition was calculated[22].

Inhibition (%) = Test - Control / Control X 100

Tail clip Treatment was carried out as;

Group I:Control (Saline received)

Group II:Standard (Pentazocin 5 mg/kg received)

Group III:Test 1 (Extract of 100 mg/kg received)

Group IV:Test 2 (Extract of 200 mg/kg received)

RESULTS

Table.1. Results of qualitative phytochemical screening of hydroalcoholic extract of *Strobilanthes ciliatus* Nees

Phytoconstituents	Chemical test	Result
Alkaloids	Dragendorff's Test Mayer's Test Hager's Test Wagner's Test	_
Saponins Glycosides	Foaming test Hemolytic test	+
Carbohydrates	Molisch test Feling's test	+
Tannins	Ferric chloride Lead acetate test	+
Flavonoids	Shinoda's test	+
Sterols	Salkowski's test Libermann-Burchard Test	+
Proteins and Amino acids	Biuret Test Ninhydrin Test	_
Terpenoids	Noller's test	+
Fixed Oils and Fats	Spot Test	_
Gums and Mucilage	Precipitate formation in alcohol	_

(+): Present, (-): Absent

Table.2. Calibration table of Quercetin (Std)

Con. µg/ml	Absorption
5	0.0384
10	0.0769
25	0.1608
50	0.3304
100	0.6704

Table.3. Total percentage content of selective phytoconstituents of *Strobilanthes ciliatus* Nees

Crude powder drug	Steroidal Saponins	Tannins	Flavonoids	Terpenoids
Leaf	28.4%	11.69%	5.2%	12.23%
Root	29.6%	10.85%	3.1%	13.26%
Stem	19.3%	7.93%	2.4%	8.54%

Table.4. Linear equation, R^2 and IC_{50} values of standard drug and hydroalcoholic extract of *Strobilanthes ciliatus* Nees

S.No	Sample	Linear equation	R^2	IC_{50} value
1	Std (Methotrexate)	Y = 61.70*X - 12.96	0.9003	3.31µg/ml
2	Extract	Y = 82.69*X - 72.67	0.9560	3.68 µg/ml

Table.5. Analgesic effect of *Strobilanthes ciliatus* Nees on tail clip method

S.No	Group	Response for noxious stimuli			
		0min	30min	60min	90min
1	**Control**	2.21±0.06	3.15±0.18	3.21±0.21	2.37±0.13
2	**Standard**	2.44±0.12	11.12±0.23*** (71.6%)	12±0.1113*** (73.25%)	10.94±0.32*** (78.33
3	**Test 1**	2.32±0.13	4.7±0.32*** (32.9%)	5.8±0.08*** (44.65%)	5.6±0.08*** (57.67%)
4	**Test 2**	2.27±0.08	4.87±0.02*** (35.3%)	6.26±0.43*** (48.72%)	7.14±0.10*** (66.8%)

Values are reported as mean ± S.E.M. for group of seven animals. The data was analyzed by two way ANOVA followed by Bonferroni post test. ***p <0.001 when compared to vehicle control.

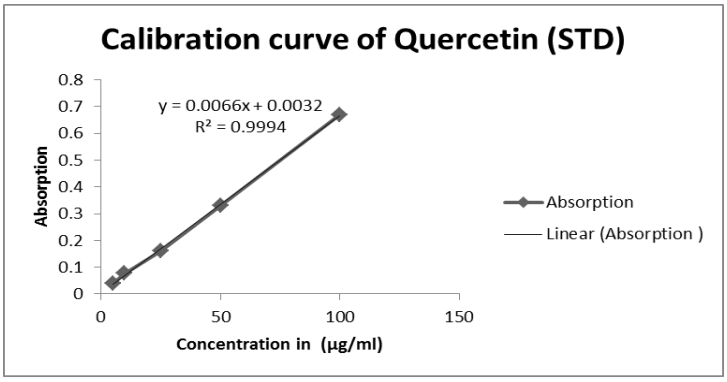

Fig.1.Calibration curve of Quercetin

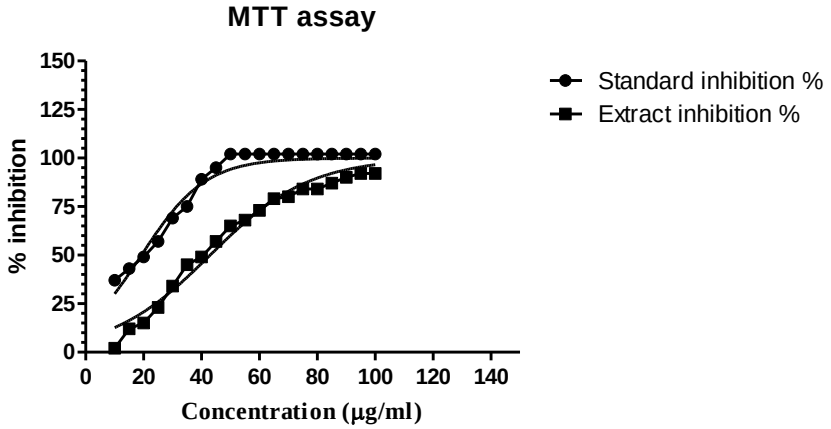

Fig.2. Cytotoxic activity of standard and extract of *Strobilanthes ciliatus* Nees against MCF-7 breast cancer cell line

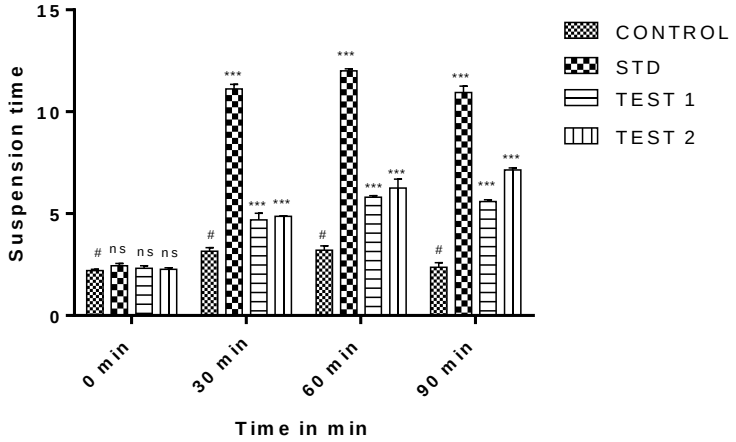

Fig.3. Analgesic effect of extract of *Strobilanthes ciliatus* Nees on tail clip method

The data were analyzed by two way ANOVA followed by a Bonferroni post test. ***P<0.001 when compared to vehicle control. Experimental data are shown as mean ± S.E.M. (n=7).

DISCUSSION

In the present work attempts were made to study detail phytochemical and biological investigations, particularly cytotoxic and analgesic property of the *Strobilanthes Ciliatus* Nees belonging to the family of Acanthaceae. Phytochemical screening provides the detail on the availability of secondary metabolites in *Strobilanthes ciliatus* Nees such as saponin glycosides, sterols, tannins, flavonoids and terpenoids (Table No.1). The total percentage content of steroidal saponins, tannins, favanoids and terpenoids were shown in the Table No.3. The root and leaf showed the maximum percentage of steroidal saponins, tannins, favanoids and terepenoids than the stem portion. Also, the results revealed that the leaves and root contain maximum content of steroidal saponins (28.4% and 29.6%) than the other phytoconstituents. The total percentage content of steroidal saponins in leaf, root and stem was 28.4%, 29.6% and 19.3% respectively. The terpenoids, tannins and flavanoids were in the range of 12.23% - 8.54%, 11.69% - 7.93% and 5.2% - 2.4%. From the qualitative and quantitative studies the entire plant was recognized as medicinally potent. The *in vitro* cytotoxic effect of various concentrations of hydroalcoholic extract of *Strobilanthes ciliatus* Nees and standard drug Methotrexate were analyzed on MC7-breast cancer cell line (Figure No.2). The concentrations that induced 50% inhibition of cell growth (IC_{50}) in µg/ml, R^2 value and linear equations are reported in Table No.4.

The cytotoxicity exhibited by the standard drug and the extracts were 3.31 µg/ml & 3.68 µg/ml. The *in vitro* studies proved the anticancer property of extract with reference to standard drug Methotrexate against MCF-7 breast cancer cell line. In acute oral toxicity studies, there was no mortality observed in rats, i.e. extract was non-toxic up to the maximum dose when administered orally. The i*n vivo* analgesic activity of the extract of 100 mg/kg produced 32.9%, 44.65% and 57.67% of inhibition of tail clipping at 30, 60 and 90 minute interval and the extract of 200 mg/kg produced 35.39%, 48.72% and 66.8% of inhibition of tail clipping at 30, 60 and 90 minute interval. The standard drug Pentazocin 5mg/kg produced 71.6%, 73.25% and 78.33% of inhibition of tail clipping at 30, 60 and 90 minutes (Table No.5 & Figure No.3) interval. The extract of 100 and 200 mg/kg produced more significant ($p<0.001$) increase in the mean latency of biting of the tail clip after 30 min and was dose dependent. The statistical data also proved that the extracts were more efficacious and comparable to that of standard Pentazocin.

CONCLUSION

The present study enabled us to conclude that the hydralcoholic extract of *Strobilanthes ciliatus* Nees has shown moderate *in vitro* cytotoxic effect. On the other hand, the extract showed more significant analgesic activity. Several plant species rich in flavonoids, steroids, saponins, terpenoids and tannins are reported to have disease preventive and therapeutic properties[23]. The cytotoxic and analgesic property recorded in the present study is in accordance with this finding, since the phytochemical evaluation indicated the presence of above phytoconstituents with promising activity. Extensive research is needed to determine the

individual component responsible for the cytotoxic & analgesic activities and molecular mechanism responsible for the same.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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